APS

(FILE 'USPAT' ENTERED AT 08:33:53 ON 05 MAY 1998)
L1 29 S (ISOPENTENYL DIPHOSPHATE OR DIMETHYLALLYL DIPHOSPHATE OR GE
L2 85 S FARNESYL DIPHOSPHATE
L3 85 S L1 OR L2
L4 0 S L3 AND PRENYL DIPHOSPHATE SYNTHASE#
L5 2 S L3 AND DIPHOSPHATE SYNTHASE#
L6 2 S DIPHOSPHATE SYNTHASE#
L7 2 S L5 OR L6

FILE 'JPO' ENTERED AT 08:36:57 ON 05 MAY 1998
L8 2 S L7

FILE 'EPOABS' ENTERED AT 08:37:15 ON 05 MAY 1998
L9 3 S L7

1. US 05443978A, Aug. 22, 1995, Chrysanthemyl **diphosphate** **synthase**, corresponding genes and use in pyrethrin synthesis; SUZANNE R ELLENBERGER, et al., C12N 9/10; C12N 15/54

US 05443978A L9: 1 of 3

DATE FILED: Jun. 25, 1993

ABSTRACT:

This invention provides a purified chrysanthemyl **diphosphate** **synthase** (CDS), a method for the purification of CDS from Chrysanthemum cinerariaefolium, and an amino acid sequence of the isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide sequence of the CDS gene, and a derived amino acid sequence of the encoded CDS protein. The CDS gene is useful in the enzymatic production of the natural stereospecific configuration of chrysanthemyl derivatives which are useful for the synthesis of pyrethrins, pyrethroids, derivatives thereof, as well as other classes of metabolites.

2. EP 00674000A2, Sep. 27, 1995, Geranylgeranyl **diphosphate** **synthase** and DNA coding therefor.; TOKUZO C O TOYOTA JIDO NISHINO, et al., C12N 9/10; C12N 15/54

EP 00674000A2 L9: 2 of 3

DATE FILED: Mar. 23, 1995

ABSTRACT:

    DNA coding for thermostable geranylgeranyl diphosphate (GGDP) synthase derived from Sulfolobus acidocaldarius is provided. The DNA is useful for production of GGDP synthase, which is, in turn, useful for production of GGDP. <IMAGE>

3. WO 09500634A1, Jan. 5, 1995, CHRYSANTHEMYL **DIPHOSPHATE** **SYNTHASE**, CORRESPONDING GENES AND USE IN PYRETHRIN SYNTHESIS; SUZANNE R ELLENBERGER, et al., C12N 9/10; C12N 15/54; C12N 15/03; C12N 15/04; C12N 15/05; C12N 15/06; C12N 15/07; C12N 15/28

WO 09500634A1 L9: 3 of 3

DATE FILED: Jun. 21, 1994

ABSTRACT:

This invention provides a purified chrysanthemyl **diphosphate** **synthase** (CDS), a method for the purification of CDS from Chrysanthemum cinerariaefolium, and a partial amino acid sequence of the isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide sequence of the CDS gene, and a derived amino acid sequence of the encoded CDS protein. The CDS gene is useful in the enzymatic production of the natural stereospecific configuration of chrysanthemyl derivatives which are useful for the synthesis of pyrethrins, pyrethroids, derivatives thereof, as well as other classes of metabolites.

1. JP409065878A , Mar. 11, 1997, LONG-CHAIN PRENYL **DIPHOSPHATE** **SYNTHASE**; ONUMA, SHINICHI, et al.,
INT-CL: C12N9/10; C07H21/04; C12N1/19; C12N15/09; C12P9/00

JP409065878A L8: 1 of 2

DATE FILED: Sep. 1, 1995

ABSTRACT:

PROBLEM TO BE SOLVED: To obtain a new prenyl **diphosphate** **synthase** comprising an amino acid at a specific part in a geranylgeranyl **diphosphate** **synthase** derived from Sulfolobus acidocaldarius, capable of a prenyl **diphosphate** **synthase** such as a steroid precursor, etc.

SOLUTION: This new variation type enzyme comprises at least one of Phe at the 77 position, Met at the 85 position, Val at the 99 position, Tyr at the 101 position, Phe at the 118 position, Arg at the 199 position and Asp at the 312 position replaced with another amino acid in a geranylgeranyl **diphosphate** **synthase** derived from Sulfolobus acidocaldarius and is capable of forming a ≥25C prenyl **diphosphate** **synthase**. Otherwise a new variation type enzyme is modified by replacement with, deficiency in and/or addition of one or a few amino acids, maintains the enzyme activity. The enzyme is useful for synthesizing a long-chain prenyl **diphosphate** **synthase** such as a steroid precursor, carotenoid, etc.

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2. JP407308193A , Nov. 28, 1995, GERANYLGERANYL **DIPHOSPHATE** **SYNTHASE** AND DNA ENCODING THE SAME; OOTO, TOKU, et al.,
INT-CL: C12N15/09; C12N1/21; C12P7/04
ADDITIONAL-INT-CL: C12N9/10

JP407308193A L8: 2 of 2

DATE FILED: Nov. 25, 1994

ABSTRACT:

PURPOSE: To obtain the subject new DNA encoding geranylgeranyl diphosphate (GGDP)synthase derived from Sulfolobus-acidocaldarius (ATCC 3,3909), capable of producing GGDP useful as a biosynthetic intermediate for carotenoid, diterpene, rubber, etc.

CONSTITUTION: This new DNA has an amino acid sequence of the formula, encodes geranylgeranyl diphosphate (GGDP)synthase derived from Sulfolobus- acidocaldarius (ATCC 33,909) and is capable of producing GGDP useful as a biosynthetic intermediate for carotenoid, diterpene, rubber, etc. The DNA is obtained by collecting a chromosome DNA from Sulfolobus-acidocaldarius, screening a gene library prepared by a conventional procedure and treating a plasmid recovered from a positive clone of the screening with a restriction enzyme.

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FILE 'USPAT' ENTERED AT 08:38:11 ON 05 MAY 1998

1. 5,622,779, Apr. 22, 1997, Cellular factors that regulate the expression of genes encoding proteins involved in cholesterol homeostasis and methods of using same; Roger Davis, 424/520 [IMAGE AVAILABLE]

US PAT NO: 5,622,779 [IMAGE AVAILABLE] L7: 1 of 2
DATE FILED: May 27, 1994

ABSTRACT:
The present invention provides a substantially purified cellular factor that can regulate the expression of genes that encode proteins involved in cholesterol metabolism. The invention also provides methods of obtaining the cellular factors of the invention in a substantially purified form. The invention further provides a method of using a cellular factor of the invention to reduce cholesterol levels in a subject having hypercholesterolemia comprising administering a cellular factor to the subject.

2. 5,443,978, Aug. 22, 1995, Chrysanthemyl **diphosphate** **synthase**, corresponding genes and use in pyrethrin synthesis; Suzanne R. Ellenberger, et al., 435/193, 252.3, 252.33, 320.1; 536/23.2, 23.6 [IMAGE AVAILABLE]

US PAT NO: 5,443,978 [IMAGE AVAILABLE] L7: 2 of 2
DATE FILED: Jun. 25, 1993

ABSTRACT:
This invention provides a purified chrysanthemyl **diphosphate** **synthase** (CDS), a method for the purification of CDS from Chrysanthemum cinerariaefolium, and an amino acid sequence of the isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide sequence of the CDS gene, and a derived amino acid sequence of the encoded CDS protein. The CDS gene is useful in the enzymatic production of the natural stereospecific configuration of chrysanthemyl derivatives which are useful for the synthesis of pyrethrins, pyrethroids, derivatives thereof, as well as other classes of metabolites.

STN

(FILE 'HOME' ENTERED AT 08:49:38 ON 05 MAY 1998)

FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE' ENTERED AT 08:49:51 ON 05 MAY 1998

L1 1210 S (FARNESYL DIPHOSPHATE OR ISOPENTENYL DIPHOSPHATE OR DIM
L2 389 S L1 AND DIPHOSPHATE SYNTHASE#
L3 2 S L1 AND PRENYL DIPHOSPHATE SYNTHASE#
L4 108 S L2 AND (MUTANT# OR MUTATION# OR VARIANT#)
L5 26 S L2 AND ((MUTANT# OR MUTATION# OR VARIANT#)(5A)(DIPHOSP
L6 26 S L5 OR L3
L7 15 DUPLICATE REMOVE L6 (11 DUPLICATES REMOVED)

=> d 1-15 ibib ab

L7 ANSWER 1 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1998:94627 CAPLUS
DOCUMENT NUMBER: 128:151099
TITLE: ***Mutants*** of a ***prenyl*** ***diphosphate*** ***synthase*** of Sulfolobus acidocaldarius for preparation of short-chain prenyl diphosphate
INVENTOR(S): Nakane, Hiroyuki; Oto, Akira; Onuma, Shinichi; Hirooka, Kazutake; Nishino, Tokuzou
PATENT ASSIGNEE(S): Toyota Motor Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 16 pp.
CODEN: JKXXAF

	NUMBER	DATE
PATENT INFORMATION:	JP 10033184 A2	980210 Heisei
APPLICATION INFORMATION:	JP 96-213211	960724

DOCUMENT TYPE: Patent
LANGUAGE: Japanese
AB Disclosed are ***mutants*** of a ***prenyl*** ***diphosphate*** ***synthase*** of Sulfolobus acidocaldarius prepd. by substitution and/or insertion in the Asp-rich domain in region II (Markush structure given). The reaction products of the ***prenyl*** ***diphosphate*** ***synthase*** are ***farnesyl*** ***diphosphate*** . Demonstrated was the prepn. of 5 geranyl- ***geranyl*** ***diphosphate*** ***synthase*** ***mutants*** of S. acidocaldarius by site-specific mutation, which were (1) 78-Thr.fwdarw.Phe and 81-His.fwdarw.Ala; (2) 78-Thr.fwdarw.Phe and 81-His.fwdarw.Leu; (3) 77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Phe, and 81-His.fwdarw.Leu; (4) 77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Phe, and 81-His.fwdarw.Ala; or (5) 77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Ser, 80-Val.fwdarw.Ile, 84-Ile.fwdarw.Leu, and insertion of Pro and Ser between 84-Ile and 85-Met. Use of the geranyl- ***geranyl*** ***diphosphate*** ***synthase*** ***mutants*** for the prepn. of C<15 prenyl diphosphates from the reactants such as ***isopentenyl*** ***diphosphate*** , di-Me allyl diphosphate, and ***geranyl*** ***diphosphate*** was also shown.

L7 ANSWER 2 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1998:58895 CAPLUS
DOCUMENT NUMBER: 128:125293
TITLE: ***Mutants*** of ***prenyl*** ***diphosphate*** ***synthase*** for preparation of long-chain prenyl diphosphate
INVENTOR(S): Oto, Toku; Ishida, Chika; Takeuchi, Yoshie; Narita, Hiroyuki; Onuma, Shinichi; Nishino, Tokuzo
PATENT ASSIGNEE(S): Toyota Motor Corp., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 17 pp.
CODEN: JKXXAF

	NUMBER	DATE
PATENT INFORMATION:	JP 10014567 A2	980120 Heisei
APPLICATION INFORMATION:	JP 96-191635	960703

DOCUMENT TYPE: Patent
LANGUAGE: Japanese
AB A ***mutant*** of ***prenyl*** ***diphosphate*** ***synthase*** is prepd. by substituting the residue that is 5-residue upstream from the Asp-rich domain I DDXX(XX)D (D=Asp; X in (XX) may not exist) in the 2nd conserved region. The synthase may be ***farnesyl*** ***diphosphate*** ***synthase*** , geranyl- ***geranyl*** ***diphosphate*** ***synthase*** , hexaprenyl ***diphosphate*** ***synthase*** , heptaprenyl ***diphosphate*** ***synthase*** , octaprenyl ***diphosphate*** ***synthase*** , nonaprenyl ***diphosphate*** ***synthase*** , or undecaprenyl ***diphosphate*** ***synthase*** . The mutant is able to catalyze the synthesis of long-chain (C>20) prenyl diphosphate. Prepn. of ***mutants*** of ***farnesyl*** ***diphosphate*** ***synthase*** (FPP synthase) of Bacillus stearothermophilus by substituting 81-Tyr with Asn, Ile, Met, Pro, Phe, and Val, resp., was shown.

L7 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1998 BIOSIS
ACCESSION NUMBER: 97:154807 BIOSIS
DOCUMENT NUMBER: 99454010
TITLE: Conversion from archaeal geranylgeranyl ***diphosphate*** ***synthase*** to ***farnesyl*** ***diphosphate*** ***synthase*** : Two amino acids before the first aspartate-rich motif solely determine eukaryotic ***farnesyl*** ***diphosphate*** ***synthase*** activity.
AUTHOR(S): Ohnuma S-I; Hirooka K; Ohto C; Nishino T
CORPORATE SOURCE: Dep. Biochemistry Engineering, Tohoku Univ., Aoba Aramaki, Aoba-ku, Sendai 980-77, Japan
SOURCE: Journal of Biological Chemistry 272 (8). 1997. 5192-5198. ISSN: 0021-9258
LANGUAGE: English
AB ***Farnesyl*** ***diphosphate*** (FPP) and geranylgeranyl

diphosphate (GGPP) are precursors for a variety of important natural products, such as sterols, carotenoids, and prenyl quinones. Although FPP synthase and GGPP synthase catalyze similar consecutive condensations of ***isopentenyl*** ***diphosphate*** with allylic diphosphates and have several homologous regions in their amino acid sequences, nothing is known about how these enzymes form the specific products. To locate the region that causes the difference of final products between GGPP synthase and FPP synthase, we constructed six mutated archaeal GGPP synthases whose regions around the first aspartate-rich motif were replaced with the corresponding regions of FPP synthases from human, rat, Arabidopsis thaliana, Saccharomyces cerevisiae, Escherichia coli, Bacillus stearothermophilus, and from some other related mutated enzymes. From the analysis of these mutated enzymes, we revealed that the region around the first aspartate-rich motif is essential for the product specificity of all FPP synthases and that the mechanism of the chain termination in eukaryotic FPP synthases (type I) is different from those of prokaryotic FPP synthases (type II). In FPP synthases of type I, two amino acids situated at the fourth and the fifth positions before the motif solely determine their product chain length, while the product specificity of the type II enzymes is determined by one aromatic amino acid at the fifth position before the motif, two amino acids inserted in the motif, and other modifications. These data indicate that FPP synthases have evolved from the progenitor corresponding to the archaeal GGPP synthase in two ways.

L7 ANSWER 4 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1997:170512 CAPLUS
DOCUMENT NUMBER: 126:183141
TITLE: Crystal structure of recombinant avian wild type and ***mutant*** ***farnesyl*** ***diphosphate*** ***synthase*** and the binding modes of substrates and inhibitor compounds (prenyltransferases)
AUTHOR(S): Tarshis, Larry
CORPORATE SOURCE: Yeshiva Univ., New York, NY, USA
SOURCE: (1996) 486 pp. Avail.: Univ. Microfilms Int., Order No. DA9705286
From: Diss. Abstr. Int., B 1997, 57(9), 5632
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L7 ANSWER 5 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1996:635257 CAPLUS
DOCUMENT NUMBER: 125:269265
TITLE: ***Mutant*** ***farnesyl*** ***diphosphate*** ***synthase*** genes of Bacillus stearothermophilus, their preparation, and use in synthesizing geranylgeranyl diphosphate
INVENTOR(S): Ayumi, Koike; Tokuzo, Nishino; Shusei, Obata; Shinichi, Ohnuma; Takeshi, Nakazawa; Kyozo,

Ogura; Tanetoshi, Koyama
PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan
SOURCE: Eur. Pat. Appl., 50 pp.
CODEN: EPXXDW

	NUMBER	DATE
PATENT INFORMATION:	EP 733709 A2	960925
DESIGNATED STATES:	R: BE, CH, DE, FR, GB, IT, LI, SE	
APPLICATION INFORMATION:	EP 95-115423	950929
PRIORITY APPLN. INFO.:	JP 95-25253	950214

DOCUMENT TYPE: Patent
LANGUAGE: English
AB The genes encoding 4 ***farnesyl*** ***diphosphate*** ***synthase*** ***mutants*** were prepd. by treating the wild type gene of Bacillus stearothermophilus with Na nitrite. These mutants have .gtoreq.1 mutations at position 34, 59, 81, 157, 182, 239, or 275 corresponding the wild type ***farnesyl*** ***diphosphate*** ***synthase*** . The mutated enzymes were produced in transgenic Escherichia coli. The mutants produced an amt. of geranylgeranyl diphosphate more than that of ***farnesyl*** ***diphosphate*** .

L7 ANSWER 6 OF 15 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 96324966 MEDLINE
DOCUMENT NUMBER: 96324966
TITLE: Conversion of product specificity of archaebacterial geranylgeranyl- ***diphosphate*** ***synthase*** . Identification of essential amino acid residues for chain length determination of prenyltransferase reaction.
AUTHOR: Ohnuma S; Hirooka K; Hemmi H; Ishida C; Ohto C; Nishino T
CORPORATE SOURCE: Department of Biochemistry and Engineering, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai 980-77, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 2) 271 (31) 18831-7.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199611
AB Prenyltransferases catalyze the consecutive condensation of ***isopentenyl*** ***diphosphate*** with allylic diphosphates to produce prenyl diphosphates whose chain lengths are absolutely determined by each enzyme. To investigate the mechanism of the consecutive reaction and the determination of the ultimate chain length, a random mutational approach was planned. A geranylgeranyl- ***diphosphate*** ***synthase*** gene from Sulfolobus acidocaldarius was randomly mutagenized by NaNO2 treatment to construct a library of mutated geranylgeranyl- ***diphosphate*** ***synthase*** genes on a yeast expression vector. The library was

screened for suppression of a pet phenotype of yeast C296-LH3, which is deficient in hexaprenyl- ***diphosphate*** ***synthase*** . Five ***mutants*** that could grow on a YEPG plate, which contained only glycerol as an energy source instead of glucose, were selected from approximately 1,400 mutants. All selected mutated enzymes catalyzed the formation of polyprenyl diphosphates with prenyl chains longer than geranylgeranyl diphosphate. Especially mutants 1, 3, and 5 showed the strongest elongation activity to produce large amounts of geranylfarnesyl diphosphate with a concomitant amount of hexaprenyl diphosphate. Sequence analysis revealed that each mutant contained a few amino acid substitutions and that the mutation of Phe-77, which is located on the fifth amino acid upstream from the first aspartate-rich consensus motif, is the most effective for elongating the ultimate product. Amino acid alignment of known prenyltransferases around this position and our previous observations on ***farnesyl*** - ***diphosphate*** ***synthase*** (Ohnuma, S.-i., Nakazawa, T., Hemmi, H., Hallberg, A.-M., Koyama, T., Ogura, K., and Nishino, T.(1996) J. Biol. Chem. 271, 10087-10095) clearly indicate that the amino acid at the position of all prenyltransferases must regulate the chain elongation.

L7 ANSWER 7 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 2
ACCESSION NUMBER: 1996:388315 CAPLUS
DOCUMENT NUMBER: 125:52249
TITLE: Identification of significant residues in the substrate binding site of Bacillus stearothermophilus ***farnesyl*** ***diphosphate*** ***synthase***
AUTHOR(S): Koyama, Tanetoshi; Tajima, Masaya; Sano, Hiroaki; Doi, Takashi; Koike-Takeshita, Ayumi; Obata, Shusei; Nishino, Tokuzo; Ogura, Kyozo
CORPORATE SOURCE: Faculty of Engineering, Tohoku University, Sendai, 980-77, Japan
SOURCE: Biochemistry (1996), 35(29), 9533-9538
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CJACS-IMAGE; CJACS
AB ***Farnesyl*** ***diphosphate*** ***synthase*** (I) from a wide range of organisms was previously shown to possess 7 highly conserved regions (I-VII) in the amino acid sequence. Site-directed mutants of I from B. stearothermophilus were made to evaluate the roles of the conserved Asp residues in region VI and Lys residues in regions I, V, and VI. Asp-224 was changed to Ala or Glu (mutants D224A and D224E, resp.); Asp-225 and Asp-228 were changed to Ile and Ala (D225I and D228A, resp.); Lys-238 was changed to either Ala or Arg (K238A or K238R, resp.). Lys-47 and Lys-183 were changed to Ile and Ala (K47I and K183A, resp.). Kinetic analyses of the wild-type and mutant enzymes indicated that mutagenesis of Asp-224 and Asp-225 resulted in a decrease in kcat values of approx. 104- to 105-fold compared to wild-type I. On the other hand, D228A showed a kcat .apprx.1/10 of that of wild-type I, and the Km for ***isopentenyl*** ***diphosphate*** increased .apprx.10-fold.

Both K47I and K183A mutants exhibited Km values for ***isopentenyl*** ***diphosphate*** 20-fold larger and kcat values 70-fold smaller than did wild-type I. These results suggest that the 2 conserved Lys residues in regions I and V contribute to the binding of ***isopentenyl*** ***diphosphate*** and that the 1st and the 2nd Asp residues in region VI are involved in catalytic function. Asp-228 is also important for the binding of ***isopentenyl*** ***diphosphate*** rather than for the catalytic reaction.

L7 ANSWER 8 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1996:703698 CAPLUS
DOCUMENT NUMBER: 126:16162
TITLE: Substrate specificities of wild and mutant FPP synthases from Bacillus stearothermophilus
AUTHOR(S): Maki, Y.; Shimizu, K.; Arai, H.; Ono, H.; Koyama, T.; Ogura, K.
CORPORATE SOURCE: Department Chemistry, Yamagata University, Yamagata, 990, Japan
SOURCE: Tennen Yuki Kagobutsu Toronkai Koen Yoshishu (1996), 38th, 295-300
CODEN: TYKYDS
PUBLISHER: Nippon Kagakkai
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB Substrate specificity of FPP synthases (I) of B. stearothermophilus is studied with several synthetic substrates. The substrate specificity is very similar to that of chicken liver and pig liver. The cysteine residues in position-73 and -289 are not assocd. with the enzymic activity. However, the Gln residue in position-221 is involved in the binding of allylic substrates and catalysis of I.

L7 ANSWER 9 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 3
ACCESSION NUMBER: 1994:403883 CAPLUS
DOCUMENT NUMBER: 121:3883
TITLE: Yeast ***farnesyl*** - ***diphosphate*** ***synthase*** : Site-directed mutagenesis of residues in highly conserved prenyltransferase domains I and II
AUTHOR(S): Song, Linsheng; Poulter, C. Dale
CORPORATE SOURCE: Dep. Chem., Univ. Utah, Salt Lake City, UT, 84112, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(8), 3044-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Prenyltransferases that catalyze the fundamental chain elongation reactin in the isoprenoid biosynthetic pathway contain several highly conserved amino acids, including two aspartate-rich regions thought to be involved in substrate binding and catalysis. The authors report a study of site-directed ***mutants*** for yeast ***farnesyl*** - ***diphosphate*** ***synthase*** (FPPSase; ***geranyl*** - ***diphosphate*** : ***isopentenyl*** -

diphosphate , EC 2.5.1.10), a prenyltransferase that catalyzes the sequential 1'-4 coupling of ***isopentenyl*** ***diphosphate*** (IPP) with ***dimethylallyl*** ***diphosphate*** and ***geranyl*** ***diphosphate*** . A recombinant form of FPPSase extended by a C-terminal -Glu-Glu-Phe .alpha.-tubulin epitope (EEF in single-letter amino acid code) was engineered to facilitate rapid purifn. of the enzyme by immunoaffinity chromatog. and to remove traces of contaminating activity from wild-type FPPSase in the Escherichia coli host. Ten site-directed mutants were constructed in FPPSase::EEF. The six aspartates in domain I (at positions 100, 101, and 104) and domain II (at positions 240, 241, and 244) were changed to alanine (mutants designated D100A, D101A, D104A, D240A, D241A, and D244A); three arginine residues were changed, Arg-109 and Arg-110 to glutamine and Arg-350 to alanine (mutants designated R109Q, R110Q, and R350A); and Lys-254 was converted to alanine (mutant designated K254A). Mutations of the aspartate residues and nearby arginine residues in domain I and Asp-240 and Asp-241 in domain II drastically lowered the catalytic activity of FPPSase::EEF. The D244A and K254A mutants were substantially less active, while kcat and the Michaelis consts. for the R350A mutant were similar to those of FPPSase::EEF. Addn. of an -EEF epitope to the C terminus of wild-type FPPSase resulted in a 14-fold increase of KmIPP and a 12-fold decrease of kcat, suggesting that the conserved hydrophilic C terminus of the enzyme may have a role in substrate binding and catalysis.

L7 ANSWER 10 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1994:403855 CAPLUS
DOCUMENT NUMBER: 121:3855
TITLE: Site-directed mutagenesis of ***farnesyl*** ***diphosphate*** ***synthase*** ; effect of substitution on the three carboxyl-terminal amino acids
AUTHOR(S): Koyama, Tanetoshi; Saito, Kazuhiro; Ogura, Kyozo; Obata, Shusei; Takeshita, Ayumi
CORPORATE SOURCE: Inst. Chem. React. Sci., Tohoku Univ., Sendai, 980, Japan
SOURCE: Can. J. Chem. (1994), 72(1), 75-9
CODEN: CJCHAG; ISSN: 0008-4042
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Site-directed mutation was introduced into the gene for the ***farnesyl*** ***diphosphate*** ***synthase*** of Bacillus stearothermophilus. To investigate the significance of the three C-terminal amino acids, where arginine is completely conserved throughout the ***farnesyl*** ***diphosphate*** ***synthases*** of prokaryotes and eukaryotes, three kinds of mutant enzymes, R295V, D296G, and H297L, which have replacements of arginine-295 with valine, aspartate-296 with glycine, and histidine-297 with leucine, resp., were overproduced and purified to homogeneity. All of the three mutant enzymes showed similar catalytic activities to that of the wild-type enzyme, indicating that the basic amino acids including the conserved arginine in the C-terminal region are not essential for catalytic function. They

were also similar to the wild-type enzyme with respect to pH optima, thermostability, reaction product, and kinetic parameters for allylic substrates. However, their Km values for ***isopentenyl*** ***diphosphate*** are approx. twice that of the wild type.

L7 ANSWER 11 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1993:644327 CAPLUS
DOCUMENT NUMBER: 119:244327
TITLE: Effect of site-directed mutagenesis of conserved aspartate and arginine residues upon ***farnesyl*** ***diphosphate*** ***synthase*** activity
AUTHOR(S): Joly, Alison; Edwards, Peter A.
CORPORATE SOURCE: Dep. Biol. Chem., Univ. California, Los Angeles, CA, 90024, USA
SOURCE: J. Biol. Chem. (1993), 268(36), 26983-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB All polyprenyl synthases catalyze the condensation of the allylic substrate, ***isopentenyl*** ***diphosphate*** , with a specific homoallylic diphosphate substrate. Polyprenyl synthases from Homo sapiens, R. rattus, Escherichia coli, Saccharomyces cerevisiae, N. crassa, and E. herbicola contain two conserved "aspartate-rich domains" (Ashby, M. N., and Edwards, P. A. (1992) J. Biol. Chem. 267, 4128-4136). In order to det. the importance of these domains in catalysis, the conserved aspartates or arginines in domains I and II of rat ***farnesyl*** ***diphosphate*** ***synthase*** were individually mutated to glutamate or lysine, resp. The putative "active site" arginine (Brems, D. N., Breunger, E., and Rilling, H. C. (1981) Biochem. 20, 3711-3718) was mutated to lysine. Each mutant enzyme was overexpressed in E. coli and purified to apparent homogeneity. Detailed kinetic analyses of the wild type and mutant enzymes indicated that mutagenesis of Asp104, Asp107, Arg112, Arg113, and Asp243 resulted in a decreased Vmax of approx. 1000-fold compared to wild type. However, no significant change in the Km values for either the ***isopentenyl*** ***diphosphate*** or ***geranyl*** ***diphosphate*** substrate were obsd. The results strongly suggest that these amino acids, and to a lesser extent Asp244, are involved in either the condensation of ***isopentenyl*** ***diphosphate*** and ***geranyl*** ***diphosphate*** to form ***farnesyl*** ***diphosphate*** and/or the release of the ***farnesyl*** ***diphosphate*** product from ***farnesyl*** ***diphosphate*** ***synthase*** . The conservation of these amino acid residues in different enzymes from several species suggests that these domains play a similar role in other polyprenyl synthases.

L7 ANSWER 12 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1993:423605 CAPLUS
DOCUMENT NUMBER: 119:23605
TITLE: Characterization of a lysine-to-glutamic acid

mutation in a conservative sequence of ***farnesyl*** ***diphosphate*** ***synthase*** from Saccharomyces cerevisiae

AUTHOR(S): Blanchard, Laurence; Karst, Francis

CORPORATE SOURCE: Inst. Biol. Mol. Ing. Genet., Univ. Poitiers, Poitiers, 86022, Fr.

SOURCE: Gene (1993), 125(2), 185-9
CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The mutant gene erg20-2 was isolated from a yeast strain defective in ***farnesyl*** ***diphosphate*** ***synthase*** (I). This strain had the unusual property of excreting prenyl alcs., such as geraniol. The nucleotide (nt) sequence, compared with that of the wild-type gene, showed a single nt change, resulting in a Lys-197 .fwdarw. Glu substitution in I which was directly involved in terpenic alc. formation. In addn., disruption of ERG20 revealed that in yeast no other prenyltransferase is able to synthesize the ***farnesyl*** ***diphosphate*** mols. required for essential nonsterol metabolites.

L7 ANSWER 13 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1992:587237 CAPLUS

DOCUMENT NUMBER: 117:187237

TITLE: Effects of site-directed mutagenesis of the highly conserved aspartate residues in domain II of ***farnesyl*** - ***diphosphate*** ***synthase*** activity

AUTHOR(S): Marrero, Pedro F.; Poulter, C. Dale; Edwards, Peter A.

CORPORATE SOURCE: Dep. Biol. Chem., UCLA, Los Angeles, CA, 90024, USA

SOURCE: J. Biol. Chem. (1992), 267(30), 21873-8
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Comparison of the ***farnesyl*** ***diphosphate*** ***synthase*** (I) amino acid sequences from 4 species with amino acid sequences from the related enzymes, hexaprenyl ***diphosphate*** ***synthase*** and geranylgeranyl diphosphate (GPP) synthase showed the presence of 2 aspartate-rich highly conserved domains. The aspartate motif [(I, L, or V)XDDXXD] of the 2nd of those domains exhibited homol. with at least 9 prenyl-transfer enzymes that utilize an allylic prenyl diphosphate as 1 substrate. In order to investigate the role of this 2nd aspartate-rich domain in rat I, the 1st or 3rd aspartate was mutated to glutamate, the wild-type and mutant enzymes were expressed in Escherichia coli, and the enzymes were purified to apparent homogeneity using a single chromatog. step. Approx. 12 mg of homogeneous protein was isolated from 120 mg of crude bacterial ext. The kinetic parameters of purified wild-type recombinant I contg. the DDYLD motif were as follows: Vmax = 0.84 .mu.mol/min/mg; GPP Km = 1.0 .mu.M; ***isopentenyl*** ***diphosphate*** (IPP) Km = 2.7 .mu.M. Substitution of glutamate for the 1st aspartate (EDYLD)

decreased the Vmax by >90-fold. The Km for IPP increased, whereas the Km for GPP remained the same in this D243E mutant. Substitution of glutamate for the 3rd aspartate (DDYLE) did not result in altered enzyme kinetics in the D247E mutant. These results suggest that the 1st aspartate (Asp-243) in the 2nd domain is involved in the catalysis by I.

L7 ANSWER 14 OF 15 CAPLUS COPYRIGHT 1998 ACS
ACCESSION NUMBER: 1990:96998 CAPLUS
DOCUMENT NUMBER: 112:96998
TITLE: Process for obtaining terpenic aromas by a microbiological process
INVENTOR(S): Karst, Francis; Vladescu, Barbu Dinu Vladimir
PATENT ASSIGNEE(S): Pernod-Ricard S. A., Fr.
SOURCE: Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW

	NUMBER	DATE
PATENT INFORMATION:	EP 313465 A1	890426
DESIGNATED STATES:	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE	
APPLICATION INFORMATION:	EP 88-402647	881020
PRIORITY APPLN. INFO.:	FR 87-14609	871022

DOCUMENT TYPE: Patent
LANGUAGE: French
AB Saccharomyces cerevisiae mutants which are blocked in the ergosterol synthetic pathway and which consequently secrete terpenoid aromas, e.g. geraniol, farnesol, linalool, are produced. Such mutants are further mutagenized to produce double mutants which are addnl. defective in alc. dehydrogenase I (ADH-I) or ADH-IIe. The former may be used to pepd. flavored fruit juice, milk, or cereal must. The latter may be used to prep. sparkling wine. Thus, a mutant deficient in squalene synthetase activity (requires ergosterol for growth) and ADH-I was prepd. This mutant, erg 9, was used to produce fruit juices with various flavors/aromas due to its prodn. of farnesol.

L7 ANSWER 15 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 4
ACCESSION NUMBER: 1990:4293 CAPLUS
DOCUMENT NUMBER: 112:4293
TITLE: Isolation and characterization of an Escherichia coli ***mutant*** having temperature-sensitive ***farnesyl*** ***diphosphate*** ***synthase***
AUTHOR(S): Fujisaki, Shingo; Nishino, Tokuzo; Katsuki, Hirohiko; Hara, Hiroshi; Nishimura, Yukinobu; Hirota, Yukinori
CORPORATE SOURCE: Fac. Gen. Educ., Gifu Univ., Gifu, 501-11, Japan
SOURCE: J. Bacteriol. (1989), 171(10), 5654-8
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The screening of a collection of highly mutagenized strains of E.

coli for defects in isoprenoid synthesis led to the isolation of a ***mutant*** that had temp.-sensitive ***farnesyl*** ***diphosphate*** ***synthase*** . The defective gene, named ispA, was mapped at about min 10 on the E. coli chromosome, and the gene order was shown to be tsx-ispA-lon. The mutant ispA gene was transferred to the E. coli strain with a defined genetic background by P1 transduction for investigation of its function. The in vitro activity of ***farnesyl*** ***diphosphate*** ***synthase*** of the ***mutant*** was 21% of that of the wild-type strain at 30.degree. and 5% of that at 40.degree.. At 42.degree. the ubiquinone level was lower (66% of normal) in the mutant than in the wild-type strain, whereas at 30.degree. the level in the mutant was almost equal to that in the wild-type strain. The polyprenyl phosphate level was slightly higher in the mutant than in the wild-type strain at 30.degree. and almost the same in both strains at 42.degree.. The mutant had no obvious phenotype